user manual
hybridization

Hybridization oven/shaker

RPN2511

Underwriters Laboratories
Inc. Listed, #E195497

GE imagination at work

Hybridization Oven/Shaker
RPN2511

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IMPORTANT: Please read the separate instructions for use before operating the GE Healthcare Hybridization Oven/Shaker to familiarize yourself with its electrical installation and safety precautions.

1.0 Introduction

One of the most widely used techniques in the molecular biology field is the immobilization of DNA and RNA onto a solid support membrane and subsequent hybridizations with a specific single stranded probe, labelled to facilitate its detection.

Using the GE Healthcare Hybridization Oven/Shaker ensures that the temperature and shaking/rotation frequency, and hence the stringency of hybridization and washing steps are rigidly controlled. This enables rapid and reproducible probing of nucleic acids, and proteins immobilized on nylon and nitrocellulose membranes.

The GE Healthcare Hybridization Oven/Shaker is a multipurpose instrument combining accurate temperature control with a choice of interchangeable hybridization modes:

- Variable speed rotisserie: holding 7 x 35 mm or 2 x 70 and 2 x 35 mm hybridization bottles.
- A variable speed platform shaker for 'box' hybridizations, depurination, denaturation and neutralization steps.

The instrument is:

- Economical: bottle hybridization minimizes probe volumes, reducing reagent volumes and enhancing signal intensity.
- Precise: stringency of hybridization and washing steps are rigidly controlled, ensuring reproducible results.
- Sensitive: validated protocols ensure optimal hybridization and washing steps, enhancing multiple reprobing when using Hybond™ membranes.
- Safe: the double-glazed polycarbonate/acrylic door offers excellent thermal insulation whilst minimizing radiation exposure.
- Convenient: small foot-print maximizes the use of limited laboratory space.

The instrument is suitable for use in conjunction with radiolabelled probes using Rapid-hyb™ buffer, non-radioactive nucleic acid labelling and detection systems such as AlkPhos Direct™, and protein labelling and detection systems including ECL™ and ECL Plus™. Some protocols for use with these applications are included in section 6.

2.0 Specification

Overall dimensions		
Height:	9.5 "	(24.0 cm)
Depth:	10.0 "	(25.0 cm)
Width:	11.25 "	(28.5 cm)

Oven dimensions		
Height:	8.0 "	(20.0 cm)
Depth:	9.0 "	(23.0 cm)
Width:	10.0 "	(25.0 cm)

Weight:	24 kg
Capacity (nominal):	18 litres
Temperature range:	Ambient plus 5 °C–80 °C
Temperature precision:	+/- 0.5 °C
Temperature fluctuation:	+/- 0.1 °C (@37°C)
Power rating:	250 W
Over temperature cut-out:	1 °C over set temperature
Temperature variation:	<0.25 °C
Rotisserie speed:	2–10 rev/min
Shaker platform speed:	5–70 strokes/min
Total angle of tilt:	4° or 10° angle

Electrical

Nominal Voltage/Hertz/Amp	Product code
230 V/50 Hz/3.1 A	RPN2510
110 V/120 V/60 Hz/4 A	RPN2511
100 V/50/60 Hz	RPN2512

Each instrument is supplied with 1 rotisserie (RPN2514), 6 hybridization bottles (RPN2516) and an instruction manual.

Accessories

Rotisserie, holds 7 x 35 mm hybridization bottles	RPN2514
Rotisserie, holds 2 x 70 mm and 2 x 35 mm hybridization bottles	RPN2515
Hybridization bottle, 230 x 35 mm (For rotisserie RPN2514, pack of 6)	RPN2516
Hybridization bottle, 150 x 35 mm (For rotisserie RPN2514, pack of 6)	RPN2517
Hybridization bottle, 230 x 70 mm (For rotisserie RPN2515, pack of 2)	RPN2518
Hybridization mesh, 1 roll 21.5 cm x 10 m	RPN2519

3.0 Setting up the Hybridization Oven/Shaker

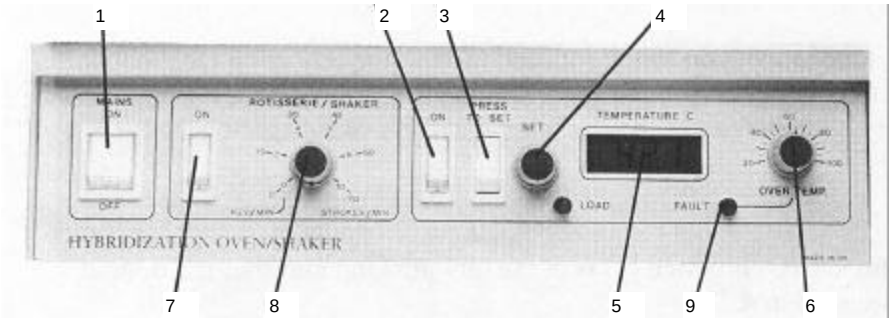
3.0.1 Remove all packaging and place the Hybridization Oven/Shaker on a level working surface, ensuring that there is sufficient room above the instrument to allow the door to be opened fully.

3.0.2 Plug the female end of the power cable into the Hybridization Oven/Shaker.

3.0.3 Connect the power cable to a suitably grounded electrical outlet. The correct operating voltage of the Hybridization Oven/Shaker is found on the product information label on the rear of the instrument.

3.0.4 Turn the Mains ON/OFF switch (1 on Fig 1) to the ON position.

3.0.5 The GE Healthcare Hybridization Oven/Shaker is now ready for use.



1. Mains ON/OFF switch
2. Temperature ON/OFF switch
3. Temperature SET switch
4. Temperature SET dial
5. Digital temperature display
6. Oven OVER TEMP dial
7. Rotisserie/shaker ON/OFF switch
8. Rotisserie/shaker speed selector dial
9. Oven FAULT LED

3.1 Fig 1. Diagram of instrument control panel

3.2 Setting the oven temperature

3.2.1 Press the Mains ON/OFF switch (1, see Fig 1) to the ON position.

3.2.2 Press the Temperature ON/OFF switch (2) to the ON position.

3.2.3 Press the Temperature SET switch (3), at the same time rotate the Temperature SET dial (4) until the required temperature is shown on the digital display (5).

3.2.4 Release the Temperature SET switch (3).

3.2.5 Rotate the Oven OVER TEMP dial (6) to the SET temperature +5 °C.

NOTE: The automatic temperature cut-out operates at 1 °C above the SET temperature, to ensure that the desired temperature is maintained at all times.

The OVER TEMP setting acts as an instrument fail safe mechanism. Should the OVER TEMP setting be reached, the red FAULT LED (9) will light up denoting a fault in the temperature control system.

3.2.6 The oven will now automatically heat up the the SET temperature.

3.3 Setting up the rotisserie

The rotisserie is installed in the Hybridization Oven/Shaker during transit. To use the rotisserie for bottle hybridization, the following procedure should be adopted:

3.3.1 Lift up the oven door to its fullest extent to allow complete access to the oven interior.

3.3.2 Lift the rotisserie vertically out of the oven and place on the bench.

3.3.3 Place the membranes to be hybridized in to the required number of hybridization bottles. Using the rotisserie as a stand for the bottles, place the bottles into the rotisserie, pushing them down as far as they will go.

NOTE: Always ensure that the weight is evenly distributed on both sides of the rotisserie. Place an empty hybridization bottle into the other side of the rotisserie as a counterbalance if necessary.

3.3.4 Place the rotisserie into the voen onto the rotation mechanism, ensuring that the serrated bands at either end of the rotisserie locate onto the steel cogs of the rotation mechanism at the rear of the oven. The plastic flanges of the rotisserie locate on to the small wheels on the oven floor. Close the oven door.

3.3.5 Ensure that the Mains ON/OFF switch (1) is in the ON position and that the desired temperature has been set (see section 4.2).

3.3.6 Turn the Rotisserie/Shaker ON/OFF switch (7) to the ON position.

3.3.7 Turn the Rotisserie/Shaker speed selector dial (8) clockwise until the desired rotation speed is reached (allowable values are 2–10 rpm).

The rotisserie will now start to rotate at the set rate.

3.3.8 When hybridization is complete turn the Rotisserie/Shaker ON/OFF switch (7) to the OFF position.

3.4 Setting up the platform shaker

During transit, the platform is stored vertically at the rear of the oven chamber. It can remain in this position whilst the rotisserie is in use. To use the platform shaker for 'sandwich box' hybridizations, the following procedure should be adopted:

3.4.1 Open the oven door to its fullest extent, lift the rotisserie vertically and store in a safe place.

3.4.2 Lift the platform by its handle (A, see figure 2) from its storage position, slide it forward and locate it on the rocking mechanism by placing the side pegs (8) of the platform into the retainers (C) on the side walls of the oven chamber. This action seats the nylon blocks (D) on the underside of the platform on to the pegs (E) protruding from the rocker mechanism at the rear of the oven.

3.4.3 Place the box in which the hybridization is being performed on to the shaker platform and close the oven door.

3.4.4 Ensure the Mains ON/OFF switch (1, see figure 1) is in the ON position and that the desired temperature has been set (see section 4.2).

3.4.5 Turn the Rotisserie/Shaker ON/OFF switch (7) to the ON position.

3.4.6 Rotate the Rotisserie/Shaker speed selector dial (8) clockwise, until the desired shaker speed is reached. Allowable values are 5–70 strokes per minute.

The shaker platform will now oscillate at the set rate.

3.4.7 When hybridization is complete, turn the ON/OFF switch (7) to the OFF position.

NOTE: The shaker platform can be operated at a choice of tilt angles, 4° or 10° The larger the angle, the greater the vertical movement of the platform at the end of each stroke, and hence the greater the agitation of the contents of the box.

This facility allows further fine-tuning of the rocking motion of the shaker platform in addition to controlling the shaker speed.

The tilt angle is controlled by the position of the pegs in the rocker mechanism, which is attached to the rear of the spill tray. To alter the tilt angle, unscrew the spill tray by rotating the nylon retaining screws anti-clockwise. Lift out the spill tray. Remove the two pegs (E in figure 2) and screw in to the appropriate position in the rocker mechanism. Figure 3 below, shows the relative positions of each hole and the tilt angle to which they correspond.

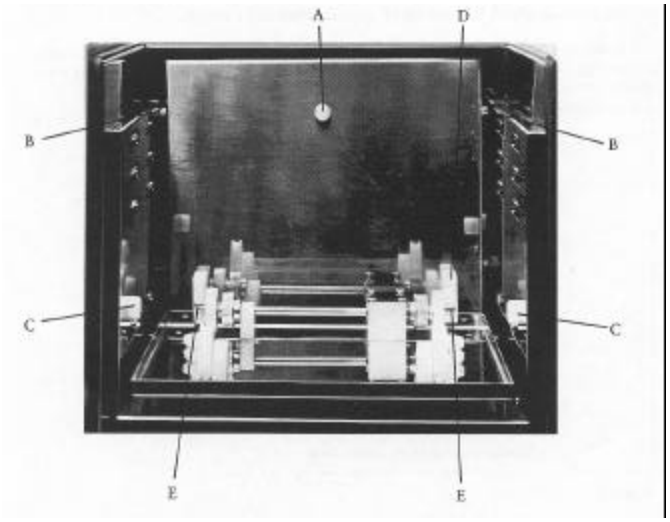


Fig 2. Hybridization oven drive components

On completion, ensure that the spill tray is repositioned correctly and screwed down securely. On starting the shaker, the drive shaft will automatically re-engage itself. This may take a few revolutions of the drive mechanism.

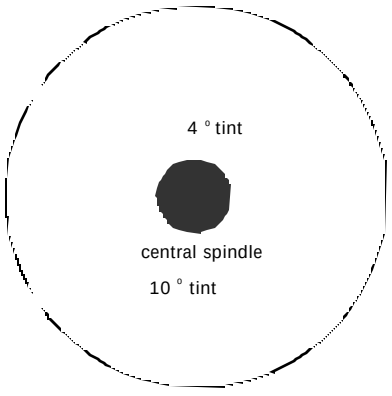


Fig 3. Adjustment of angle of tilt of platform shaker

4.0 Hybridization using the platform shaker

The Hybridization Oven/Shaker is compatible with the hybridization technologies available from GE Healthcare. These include radioactive hybridizations using Rapid hyb buffer and the range of non-radioactive systems for the labelling and detection of proteins and nucleic acids (see appendix 1).

When using the platform shaker the hybridization and washing conditions recommended in the appropriate GE Healthcare literature should be used. The following protocol therefore provides a guideline. For specific hybridization times and temperatures, refer to the relevant protocol booklet.

4.0.1 Prepare blots as recommended in the appropriate Hybond protocol booklet.

4.0.2 Set the oven temperature and over temperature values as described in section 4.2.

4.0.3 Place the membrane in a suitable box (or bag) and cover with sufficient prehybridization buffer to ensure that the entire surface of the membrane is covered. Recommended volume: surface area ratio is given in most protocol booklets. Seal the box (or bag) securely.

4.0.4 Place the box (or bag) on the platform, set the oscillation speed to 30 strokes/min and prehybridize for the required length of time.

4.0.5 Remove the box (or bag) from the oven and carefully add the labelled single stranded probe (denaturation may be required post labelling refer to the appropriate protocol booklet) to the prehybridization buffer.

NOTE: Do not pipette the probe solution directly on to the membrane as this may cause localized background.

4.0.6 Reseal the box (or bag) securely, replace it on the platform and hybridize for the required length of time.

4.0.7 Remove the membranes and place in a clean box containing the first stringency wash solution.

4.0.8 Increase the oscillation speed of the platform to 60 strokes/min. Carry out the recommended washing protocol at the appropriate temperature and for the appropriate times.

5.0 Hybridization using the rotisserie

Several advantages are associated with performing hybridizations in bottles, namely those of safety and economy as outlined in the introduction. However, the use of bottles for hybridizations and washing procedures requires certain adaptations to standard protocols.

5.1 Assembly of membranes into bottles

5.1.1 Add approximately 20 ml 2x SSC buffer into the hybridization bottle. The rotisserie acts as a convenient bottle stand.

5.1.2 Pre-wet the membrane in 2x SSC buffer and loosely roll it up.

5.1.3 Insert the rolled up membrane into the bottle and replace the cap. Ensure that the cap is screwed on securely, (hand tight plus a quarter turn). DO NOT OVERTIGHTEN, or the thread of the cap can be damaged, leading to leakages.

NOTE: If placing several small blots into one bottle, prewet the membranes as above, and space them out along the length of the bottle with forceps.

5.1.4 Place the bottle on a flat surface and roll it gently in the opposite direction to that which the membrane is coiled. This rolling action causes the membrane to uncoil, so lining the inner surface of the bottle.

NOTE: The use of a mesh in bottle hybridizations to ensure uniform contact between the membrane surface and the buffer has been recommended.

However, studies at GE Healthcare laboratories using a wide range of hybridization mesh technologies demonstrate a resulting loss of sensitivity due to partial absorption of the probe into the mesh.

A nylon mesh (RPN2519) is available as an optional extra, as it can facilitate easier handling of a number of blots and the more fragile nitrocellulose membranes. These handling advantages should be considered against the potential loss of sensitivity before use.

When a hybridization mesh is used in conjunction with the membrane, the following procedure should be adopted:

5.1.5 Pre-wet the mesh alongside the membrane in 2x SSC buffer and place the prewetted membrane on top of the mesh. The mesh should be slightly larger than the blot in all dimensions. Roll both up together, with the mesh on the outside of the membrane, and insert into the bottle as described above (6.1.4).

5.2 Hybridization

5.2.1 Set the required oven temperature and over temperature, (as detailed in section 4.2).

5.2.2 Discard the 2x SSC, used in the bottle to unroll the membrane, and replace with prehybridization buffer. Recommended volumes are provided in most GE Healthcare protocol booklets. Generally a minimum of 10–15 ml per 20 x 20 cm blot is advised. Seal the bottle, avoiding overtightening.

5.2.3 Place the hybridization bottle(s) into the rotisserie (as detailed in section 4.3) add counterbalance bottles if necessary. Place the rotisserie into the oven so that the bottles are rotating in the same direction as the membrane is rolled, see Figure 4 overleaf.

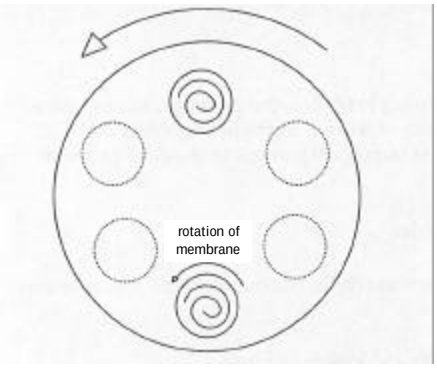


Fig 4. Rotation of rotisserie

5.2.4 Prehybridize the membrane for the specified length of time at a rotisserie speed of 4 rpm.

5.2.5 Following prehybridization, turn off the rotisserie, remove the rotisserie from the oven. Add the labelled probe to the buffer, either by removing an aliquot of the buffer, adding the probe and returning the aliquot to the bottle; or by adding the probe directly into the bottle, avoiding the membrane.

5.2.6 Hybridize for the specified length of time at a rotisserie speed of 4 rpm.

5.3 Membrane washing procedures

Membranes can either be removed from the hybridization bottles and washed in a box on the platform shaker (this is a more efficient procedure), or the washing procedure may be carried out in the bottles. If the platform shaker is used, follow the standard washing procedure mentioned in the appropriate protocol booklet.

If bottles are to be used it is necessary to modify the standard washing procedure.

Outlined in this section are the optimized washing protocols for radioactive hybridizations and non-radioactive based hybridizations.

Radioactive Hybridizations

5.3.1 Carefully drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with 2x SSC and discard.

5.3.2 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

2 x 10 min with 2x SSC, 0.1% SDS at 65 °C

1 x 15 min with 1x SSC, 0.1% SDS at 65 °C

2 x 10 min with 0.1x SSC, 0.1% SDS at 65 °C

NOTE: This last step is a high stringency wash and should be omitted if related sequences are to be probed.

(More washes over the same time period for each stringency condition can improve background).

5.3.3 Remove the membrane from the bottle, drain off excess stringency wash, wrap in SaranWrap™ and autoradiograph.

AlkPhos Direct Hybridizations

5.3.4 Drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with primary wash buffer and discard.

5.3.5 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

3 x 10 min with primary wash buffer solution at 55 °C

3 x 5 min with secondary wash buffer at room temperature

NOTE: The room temperature washes can be achieved by switching off the oven and leaving the door open whilst performing the washes or by allowing the oven to cool down to room temperature before performing the final washes.

5.3.6 Remove the membrane from the bottle and detect using the standard procedures outlined in the protocol booklet.

Gene Images Random Prime Hybridizations

5.3.7 Drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with 2x SSC and discard.

5.3.8 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

2 x 10 min with 1x SSC, 0.1% SDS at 60 °C

1 x 10 min with O.1x SSC, 0.1% SDS at 60 °C

5.3.9 Remove the membrane from the bottle and detect using the standard procedures outlined in the booklet. The detection procedure may be carried out using the shaker mode of the Hybridization Oven/Shaker.

NOTE: The room temperature washes can be achieved by switching off the oven and leaving the door open whilst performing the incubations or by allowing the oven to cool down to room temperature.

6.0 Maintenance/care/cleaning of the oven/shaker

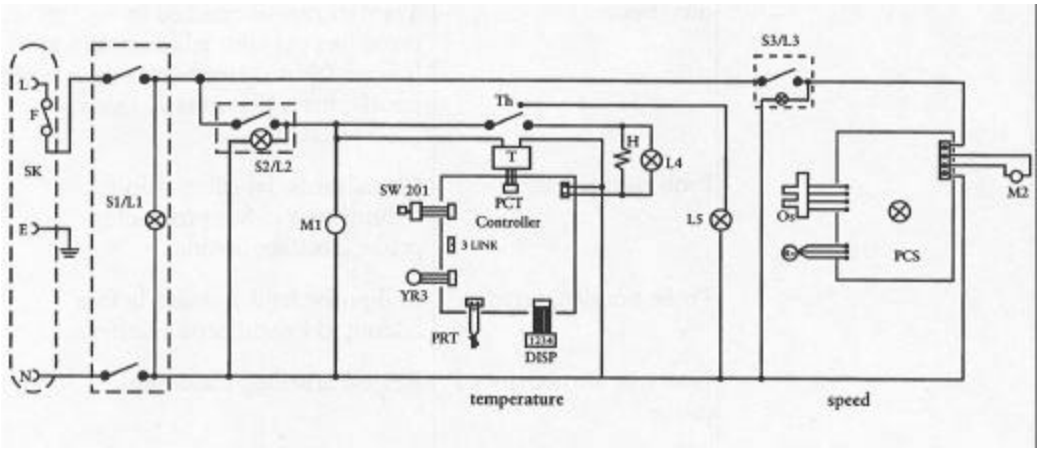
The GE Healthcare Hybridization Oven/Shaker is designed to provide trouble-free operation. The base of the oven and the shaker tray act as a spills tray and will contain any spillage that occurs during hybridization and washing procedures.

To ensure lasting operation the following instructions should be followed:

ALWAYS DISCONNECT THE HYBRIDIZATION OVEN/SHAKER FROM THE ELECTRICAL SUPPLY BEFORE CLEANING OR DRYING THE INSTRUMENT.

1. Any leakage from the hybridization bottles or the sandwich boxes should be cleaned up immediately. Do not allow any liquids to enter the drive mechanism.
2. Wipe away any liquids from inside and outside of the unit using soap and water with a soft cloth or sponge.
3. Do not allow chemicals to remain on unit surfaces.
4. Never clean unit with abrasive pads or cleaners.
5. Never clean unit with acetone or chloroform.

7.0 Wiring diagram



8.0 Troubleshooting guide

This section briefly summarizes some of the potential problems encountered during membrane hybridizations. More complete troubleshooting guides are supplied in the pack leaflet that accompany each product.

Symptom	Cause	Remedy
Membrane curling up in hybridization bottle	Incorrect orientation of bottle in rotisserie	Ensure membrane is rolled up in the same direction as the bottle is rotating (see section 6.2)
High background	Probe concentration too high	Reduce probe concentration
	Unincorporated ^{32}P nucleotides not removed	Remove unincorporated nucleotides eg. using Amprep™ C18 column
	Insufficient blocking	Use recommended hybridization buffer or extend prehybridization time
	Insufficient washing	Increase number of buffer changes during the washing stage or increase stringency of final wash
Weak signal	No transfer from gel to membrane	Load extra lanes with control DNA. Transfer can be checked by restaining gel with ethidium bromide. If large DNA fragments are detected poorly, use a depurination step (0.25 M HCl)
	Probe not labelled	Check probe labelling before hybridization. See protocol in probe labelling booklet
	Probe not denatured	Boil probe for 5 minutes before adding to hybridization buffer
	Low specific activity of probe	Review labelling conditions
	Washes too stringent	Increase buffer salt concentration and decrease temperature
Patchy backgrounds	Hybridization buffer or wash solution not evenly covering membrane	Increase volume of hybridization wash or solutions or use mesh (see section 6.1.4)
	Damaged membrane	Handle membrane carefully with forceps
High background around edge of membrane	Damaged membrane	Use clean scissors or a sharp scalpel to cut membrane

DNA Markers

Precise Sizing					Digest of Natural DNAs				Pulsed Field		
Oligonucleotide sizing markers 27-2521-01	50 Base-pair ladder 27-4005-01	100 Base-pair ladder 27-4001-01	250 Base-pair ladder 27-4006-01	KiloBase™ DNA marker 27-4004-01	λDNA-Hind III Digest 27-4048-01	X-174 RF DNA-*Hae* III Digest 27-4040-01	X-174 RF DNA-*Hae* III Digest 27-4044-01	λDNA-*Hind* III/X-174 RF DNA-*Hae* III Digest 27-4052-01	λDNA-*Hind* III/X-174 RF DNA-*Hae* III Digest 27-4054-01 and DRigest III 27-4060-01	λDNA-PFGE Markers 27-4530-01	Yeast DNA-PFGE Markers 27-4520-01 32
32[a]	500[a]	2,000[a]	2,000	10,000[a]	23,130[b]	1,057	1,353	23,130[b]	23,130[b]	970,000	1,900,000
30	450	1,900	1,750	8,000	9,416	770	1,078	9,416	9.416	921,500	1,640,000
28	400	1,800	1,500	6,000	6,557	612	872	6,557	6,557	973,000	1,120,000[d]
26	350	1,700	1,250	5,000	4,361[b]	495	603	4,361[b]	4,361[b]	824,500	1,110,000[d]
24	300	1,600	1,000	4,000	2,322	392	310	2,322	2,322	776,000	945,000
22	250	1,500	750	3,000	2,027	345	281	2,027	2,027	727,500	915,000
20	200	1,400	500	2,500	564	341	271	1,057	1,353	679,000	815,000
18	150	1,300	250	2,000	125[c]	335	234	770	1,078	630,500	785,000
16	100	1,200		1,500		297	194	612	872	582,000	745,000
14	50	1,100		1,000		291	118	564[c]	603	533,500	680,000
12		1,000		500		210	72	495	564[c]	485,000	610,000
10		900				162		392	310	436,500	555,000
8		800				79		345	281	388,000	450,000
		792						341	271	339,500	375,000.
		700						335	234	291,000	295,000
		600						297	194	242,500	225,000
		500						291	125[c]	194,000	
		400						210	118	145,500	
		300						162	72	97,000	
		200						125[c]		48,500	
		100						79			

a not necessarily the largest size fragment possible, only the largest that is readily distinguishable
b cos ends are located on these bands
c very faint band
d chromosomes VII and XV run as a single band
e may not always be visible

Marker code number	Recommended gel	Loading amount (µg/lane)	Heat before loading
27-2521-01	20% polyacrylamide/7 M urea	3 µl radiolabelled marker/lane	90 °C for 3 min
27-4005-01	2% agarose/6% polyacrylamide	2.0	No
27-4001-01	1.5% agarose	2.0	No
27-4006-01	1% agarose	2.0	No
27-4004-01	0.8% agarose	0.5	No
27-4048-01	1% agarose	0.5	60–65 °C for 2 min
27-4040-01	1% agarose	0.5	No
27-4044-01	1% agarose	0.5	No
27-4052-01	1% agarose	0.5–1.0	60–65 °C for 2 min
27-4054-01	1% agarose	0.5–1.0	60–65 °C for 2 min
27-4060-01	1% agarose	0.5–1.0	60–65 °C for 2 min

Size	Pack size	Hybond N+	Hybond XL	Hybond N	Hybond NX	Hybond Nfp	Hybond ECL	Hybond C Extra	Hybond P
82 mm	50 discs	RPN82B	RPN82S	RPN82N	RPN82T		RPN82D	RPN82E	
87 mm	50 discs	RPN87B	RPN83S	RPN87N	RPN87T				
132 mm	50 discs	RPN132B	RPN132S	RPN132N	RPN132T		RPN132D		
137 mm	50 discs	RPN137B	RPN137S	RPN137N	RPN137T		RPN137D	RPN137E	
11.9 x 7.8 cm	50 sheets	RPN119B	RPN119S	RPN119N	RPN119T				
12 x 10 cm	20 sheets	RPN1210B	RPN1210S	RPN1210N	RPN1210T				
15 x 10 cm	20 sheets	RPN1510B	RPN1510S	RPN1510N	RPN1510T				
15 x 20 cm	10 sheets	RPN1520B	RPN1520S	RPN1520N	RPN1520T		RPN1520D		
20 x 20 cm	10 sheets	RPN2020B	RPN2020S	RPN2020N	RPN2020T		RPN2020D	RPN2020E	RPN2020F
22.2 x 22.2 cm	10 sheets	RPN2222B	RPN2222S	RPN2222N	RPN2222T				
30 x 50 cm	5 sheets	RPN3050B	RPN3050S	RPN3050N	RPN3050T			RPN3050E	
20 cm x 3 m	1 roll	RPN203B	RPN203S	RPN203N	RPN203T	RPN203X	RPN203D	RPN203E	RPN303F
30 m x 3 m	1 roll	RPN303B	RPN303S	RPN303N	RPN303T				
82 mm	50 gridded discs	RPN1782B							
87 mm	50 gridded discs	RPN1787B							
132 mm	50 gridded discs	RPN1732B							
137 mm	50 discs	RPN1737B							
22.2 x 22.2 cm	50 sheets	RPN2250B							
22.5 x 22.5 cm	50 sheets	RPN225B							
	50 sheets	RPN1576B							
6 x 8 cm	50 sheets						RPN68D		
7 x 8 cm	50 sheets						RPN78D		
16 x 14 cm	15 sheets								RPN1416F
9 x 10 cm	10 sheets						RPN910D		
15 x 15 cm	10 sheets						RPN1515D		
10 x 10 cm	10 sheets						RPN1010D		
30 cm x 3 m	1 roll*						RPN3032D		

*0.2 µm pack size

Appendix 3. Radioactive labelling systems

Labelling system	Technology	Nucleotide	Amount of template	Labelling time	Probe specific activity (dpm/µg)	Recommended application
Rediprime II™	Random-Prime	dCTP only	25 ng	10 min	2×10^9	membrane hybridization
Ready-To-Go™ DNA labelling beads	Random-Prime	dCTP only	10 ng–1 µg	5 min	2×10^9	membrane hybridization
Megaprime	Random-Prime	any dNTP	25 ng	10 min	2×10^9	membrane hybridization
Nick™ translation	Nick translation	any dNTP	1 µg	2–3 hours	2×10^9	production of large amounts of probe
RNA labelling	SP6/T7 RNA polymerase	UTP	1 µg	1–2 hours	2×10^9	*in situ* hybridization
5'-end labelling	T4 polynucleotide kinase	dATP	10 pmol ends	1 hour	5×10^8	membrane hybridization *in situ* hybridization
3'-end labelling	Terminal deoxy-nucleotidyl transferase	any dNTP	10 pmol ends	30–60 min	5×10^8	membrane hybridization *in situ* hybridization

Appendix 4. Radioactive nucleotides

Compound	Concentration	Specific activity		Redivue product code	Standard product code	Pack size (see key)
		TBq/mmol	Ci/mmol			
^{32}P						
[a-^{32}P]dATP	10 mCi/ml	~220	~6000	AA0074	PB10474 (a)	1, 2 & 3
		~110	~3000	AA0004	PB10204 (a)	1, 2 & 3
		~30	~800	AA0084	PB10384 (a)	1, 2 & 3
		~15	~400	AA0064	PB10164 (a)	1, 2 & 3
[a-^{32}P]dCTP	10 mCi/ml	~220	~6000	AA0075	PB10475 (a)	1, 2 & 3
		~110	~3000	AA0005	PB10205 (a)	1, 2 & 3
		~30	~800	AA0085	PB10385 (a)	1, 2 & 3
		~15	~400	AA0065	PB10165 (a)	1, 2 & 3
[a-^{32}P]dGTP	10 mCi/ml	~110	~3000	AA0006	PB10206 (a)	1, 2 & 3
		~30	~800	AA0086	PB10386 (a)	1, 2 & 3
		~15	~400	AA0066	PB10166 (a)	1, 2 & 3
[a-^{32}P]dTTP	10 mCi/ml	~110	~3000	AA0007	PB10207 (a)	1, 2 & 3
		~30	~800	AA0087	PB10387 (a)	1, 2 & 3
		~15	~400	AA0067	PB10167 (a)	1, 2 & 3
[a-^{32}P]ATP	10 mCi/ml	~110	~3000		PB10200 (a)	1, 2 & 3
		~15	~400		PB10160 (a)	1, 2 & 3
[a-^{32}P]CTP	10 mCi/ml	~110	~3000		PB10202 (a)	1, 2 & 3
	20 mCi/ml	~30	~800		PB20382 (b)	1, 2 & 3
		~15	~400		PB10162 (a)	1, 2 & 3
	40 mCi/ml	~30	~800		PB40382 (e)	3
[a-^{32}P]GTP	10 mCi/ml	~110	~3000		PB10201 (a)	1, 2 & 3
		~15	~400		PB10161 (a)	1, 2 & 3
[a-^{32}P]UTP	10 mCi/ml	~110	~3000	AA0003	PB10203 (a)	1, 2 & 3
	20 mCi/ml	~30	~800		PB20383 (b)	1, 2 & 3
		~15	~400		PB10163 (a)	1, 2 & 3
	40 mCi/ml	~30	~800		PB40383 (e)	3
[g-^{32}P]ATP	10 mCi/ml	>185	>5000	AA0018	PB10218 (a)	1, 2 & 3
	2 mCi/ml	>185	>5000		PB218 (c)	1, 2 & 3
		~110	~3000	AA0068	PB10168 (a)	1, 2 & 3
		~110	~3000		PB168 (c)	1, 2 & 3
	10 mCi/ml	~1.11	~30		PB10132 (a)	1, 2 & 3
	2 mCi/ml	~0.11	~3.0		PB108 (c)	1, 2 & 3
	2 mCi/ml	~0.11	~3.0		PB170 (d)	1, 2 & 3
[g-^{32}P]GTP	10 mCi/ml	>185	>5000		PB10244 (a)	1 & 3
[a-^{32}P]ddATP	10 mCi/ml	>185	>5000		PB10235 (a)	1 & 3
		~110	~3000		PB10233 (a)	1 & 3
[a-^{32}P]ATP	2 mCi/ml	~1.11	~30		PB171 (d)	1, 2 & 3
[^{32}P]pCp	10 mCi/ml	~110	~3000		PB10208 (a)	1, 2 & 3
[^{32}P]NAD	10 mCi/ml	~37	~1000		PB10282 (g)	1 & 3
^{33}P						
[g-^{32}P]ATP	10 mCi/ml	37–110	1000–3000	AH9968	BF1000 (a)	1, 2 & 3
[a-^{32}P]dATP	10 mCi/ml	37–110	1000–3000	AH9904	BF1001 (a)	1, 2 & 3
[a-^{32}P]UTP	20 mCi/ml	37–110	1000–3000		BF1002 (a)	1
^{35}S						
[^{35}S]dATPaS	10 mCi/ml	>37	>1000	AG1000	SJ1304	1 & 3
		~22	~600	AG1001	SJ304	1 & 3
		~15	~400	AG1002	SJ264	1 & 3
[^{35}S]dCTPaS	10 mCi/ml	>37	>1000		SJ1305	1 & 3
		~22	>600		SJ305	
		~15	~400		SJ265	
[^{35}S]dATPaS	10 mCi/ml	>37	>1000		SJ1334 (h)	1 & 3

Compound	Concentration	Specific activity		Redivue	Standard	Pack size
		TBq/mmol	Ci/mmol	product code	product code	(see key)
[^{35}S]ATPαS		>37	>1000		SJ1300	
		~22	~600		SJ300	
		~15	~400		SJ260	
[^{35}S]CTPαS	10 mCi/ml	>37	>1000		SJ1302	1 & 3
	40 mCi/ml	~30	~800		SJ40382 (f)	3
[^{35}S]UTPαS	10 mCi/ml	>37	>1000		SJ1303	1 & 3
	20 mCi/ml	>37	>1000		SJ603 (i)	1 & 3
	10 mCi/ml	~15	~400		SJ263	1 & 3
	40 mCi/ml	~30	~800		SJ40383 (f)	3
[^{32}S]ATPγS	10 mCi/ml	>37	>1000		SJ1318	1 & 3
	10 mCi/ml	~22	~600		SJ318	1

NOTE: Redivue ^{32}P- and ^{33}P-nucleotides contain dye and stabilizer in formulation A. All ^{35}S-nucleotides are supplied in stabilized aqueous solution (containing 20 mM DTT) at 370 MBq/ml, 10 mCi/ml except where stated.

Formulation decoder

A	Stabilized aqueous solution (containing 5 mM 2-mercaptoethanol) at 370 MBq/ml, 10 mCi/ml
B	Stabilized aqueous solution (containing 5 mM 2-mercaptoethanol) at 740 MBq/ml (SP6/T7 grade)
C	Ethanol: water (1.1) now supplied in the CDC container at 74 MBq/ml, 2 mCi/ml
D	Aqueous solution at 74 MBq/ml, 2 mCi/ml
E	Stabilized aqueous solution at 1.5 GBq/ml, 40 mCi/ml (SP6/T7 grade)
F	SP6/T7 Grade supplied at 1.5 GBq/ml, 40 mCi/ml
G	Stabilized aqueous solution pH6.0
H	Contains no DTT (in situ grade)
I	Stabilized aqueous solution (containing 20 mM DTT at 740 MBq/ml, 20 mCi/ml (SP6/T7 grade)

Pack size key

1: 9.25 MBq, 250 µCi
2: 18.5 MBq, 500 µCi
3: 37 MBq, 1 mCi

Appendix 5. Non-radioactive labelling and detection systems

Labelling and detection system	Sensitivity	Time from hybridization to detection	Duration of light output	Strip before re-probing	Quantification	Recommended application
AlkPhos Direct	0.06 pg	1 hour	5 days	yes	no	Single copy Southern and Northerns
ECL Direct	0.5 pg	1 hour	1–2 hours	no	no	High target applications eg. colony/plaques
Gene Images™ Random Prime	0.1 pg	3 hours	5 days	yes	no	High sensitivity Northerns
Gene Images 3'-end labelling with CDP-Star	0.1 pg	3 hours	5 days	yes	no	Oligo screening with stringency control
Gene Images 3'-end labelling with ECF™	120 pg	3 hours	1–2 days	yes	yes	Quantification
ECL Random-Prime	0.5 pg	3 hours	1–2 hours	yes	no	Medium target Southern with DNA probes
ECL 3'-end labelling	0.2 pg	3 hours	1–2 hours	yes	no	Medium to high target Southern with oligo probes
ECF Random-Prime	0.25 pg	3 hours	1–2 days	yes	yes	Quantification

Appendix 6. Products for autoradiography and chemiluminscent detection

Hyperfilm – high performance autoradiography films

Size	Pack size	Hyperfilm MP: multipurpose film autoradiography film	Hyperfilm ECL: for use with enhanced chemiluminescence
18 x 24 cm	25 sheets	RPN6K	RPN2103K
18 x 43 cm	25 sheets	RPN36K	–
30 x 40 cm	25 sheets	RPN7K	RPN2104K
35 x 43 cm	25 sheets	RPN8K	–
35 x 43 cm	75 sheets	RPN30K	–
20 cm x 25 m	1 roll	RPN34K	–
18 x 24 inches	75 sheets	RPN1675K	RPN3103K
5 x 7 inches	25 sheets	RPN1676K	RPN1674K
8 x 10 inches	25 sheets	RPN1677K	RPN2114K
8 x 10 inches	75 sheets	RPN1678K	RPN3114K
10 x 12 inches	25 sheets	–	RPN1681K

Hypercassette™ – cassettes for autoradiography and light detection

Size	Code (standard)	Code (deep)
18 x 24 cm	RPN11642	RPN1628
24 x 30 cm	RPN11643	
30 x 40 cm	RPN11644	RPN1627
35 x 43 cm	RPN11645	
18 x 43 cm	RPN11646	
20 x 40 cm	RPN11647	
5 x 7 inches	RPN11648	
8 x 10 inches	RPN11649	RPN1629
10 x 12 inches	RPN11650	

Hyperscreen™ – intensifying screens for ^{32}P and ^{125}I autoradiography

Size	Code	Quantity
18 x 24 cm	RPN1662	1 pair
24 x 30 cm	RPN1663	1 pair
30 x 40 cm	RPN1664	1 pair
35 x 43 cm	RPN1665	1 pair
18 x 43 cm	RPN1666	1 pair
20 x 40 cm	RPN1667	1 pair
5 x 7 inches	RPN1668	1 pair
8 x 10 inches	RPN1669	1 pair
10 x 12 inches	RPN1670	1 pair

Miscellaneous

Product name	Code
Hypertorch™ Battery powered LED darkroom torch, pack of 3	RPN1620
Sensitize™ Optimized preflash system	RPN2051
TrackerTape™ An adhesive, waterproof tape that phosphoresces to give a permanent written image on film. For use with radioactive or chemiluminescent emissions	RPN2050

Appendix 7. Hybridization products

Product name	Pack size	Product code
Rapid-Hyb buffer		
Rate enhanced hybridization buffer	125 ml	RPN1635
for use with radiolabelled nucleic acid probes	500 m	RPN1636
Hybridization buffer tablets	100 tablets	RPN131
Each tablet makes 10 ml of hybridization buffer for use with any nucleic acid probe		

Appendix 8. Radiation safety products

For the safe handling and storage of ^{32}P

	Product code	Pack description
Beta shielding is manufactured from 10 mm heavy gauge, optical quality acrylic which effectively absorbs ^{32}P beta particles		
Beta starter pack – comprehensive shielding and containment package for ^{32}P users	RPN2052	1 pack
Beta safety screen, 15 ° – ideal when working seated at the bench	RPN1536	1 screen
Beta safety screen, 45 ° – when working standing up	RPN1537	1 screen
Beta side/rear screen – use vertically or horizontally to protect your colleagues	RPN2034	1 screen
Beta workbox – for the storage of low level radioactive working	RPN1539	1 box
Beta workbox insert – for the storage of 32 microcentrifuge tubes	RPN1540	1 insert
Beta safe storage box – holds 3 tube racks or workstations	RPN1541	1 box
Beta work tank – beta workstation, gives 360 ° protection from beta radiation	RPN2033	1 worktank
Beta heavy duty tube rack – 35 mm acrylic with 4 tube holes	RPN1543	1 rack
Beta waste safe – safe short-term storage of low activity waste awaiting disposal 400 x 240 x 220 mm 10 mm acrylic	RPN1532	1 safe
Beta midi waste safe – designed to hold standard 'sharps' 315 x 225 x 235 mm	RPN2038	1 safe
Beta mini waste safe – compatible with the Beta work tank 150 x 120 x 100 mm	RPN2039	1 safe
Beta tip safe – mini waste safe with hinged lid for safe pipette tip disposal 150 x 150 x 150 mm	RPN2081	1 safe
Beta tip safe plastic bags – disposable draw-string plastic bags	RPN2083	pack of 25
Beta pipette guard – designed for use with Gilson Pipetman™ range of pipettes		
for use with the P20/100 size	RPN1544	1 guard
for use with the P200 size	RPN1545	1 guard
for use with the P1000 size	RPN1546	1 guard
set of beta pipette guards (RPN1544, RPN1545, RPN1546)	RPN1556	3 guards

Radiation safety accessories

Safety tray – a defined working area for bench radioactive work		
685 x 455 mm	RPN1534	1 tray
685 x 455 mm (white**)	RPN1533	1 tray
685 x 535 mm	RPN2042	1 tray
530 x 330 mm	RPN2043	1 tray
1120 x 535 mm	RPN2063	1 tray
565 x 535 mm	RPN2083	1 tray
455 x 255mm	RPN2093	1 tray

**all other safety trays are yellow

	Product code	Pack description
Kodak™ APET safety tray liners – similar chemical resistance to above liners, but with none of the disposal problems associated with plastics such as PVC.		
to match RPN1533/34	RPN1528	pack of 25
to match RPN2042	RPN2048	pack of 25
to match RPN2043	RPN2058	pack of 25
to match RPN2063	RPN2068	pack of 25
to match RPN2083	RPN2088	pack of 25
to match RPN2093	RPN2098	pack of 25
Work box insert – compatible with work boxes and safe storage boxes		
Work box insert, 1.5 ml tubes	RPN1540	1 insert
Work box insert, 2.0 ml tubes	RPN2035	1 insert
Work box insert, 0.5 ml tubes	RPN2036	1 insert
Microcentrifuge tube rack – 'S' shaped rack with lifting points to allow easy removal from boxes		
Microcentrifuge tube rack, 1.5 ml tubes	RPN1542	1 rack
Microcentrifuge tube rack, 0.5 ml tubes	RPN2037	1 rack
Redivial station – designed to hold 2 Redivials and 16 microcentrifuge tubes	RPN1585	1 workstation
CDC storage box – lockable storage box for 4 GE Healthcare CDS's	RPN2032	1 box
Radioactive spills kit – wall mountable kit containing materials to clear up small radioactive spills	RPN2030	1 kit
Radioactive spills refill pack – refill pack for Radioactive spills RPN2030	RPN2031	1 pack

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